

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims

1. (Currently Amended) A recombinant vector ~~consisting essentially of~~ comprising (a) lactic acid bacterial DNA, ~~the vector comprising~~ (b) a gene coding for an amber suppressor ~~which~~ that is a tRNA comprising the CUA anticodon, and (c) a replicon making the vector capable of replicating in a lactic acid bacterium, wherein the vector lacks a gene coding for antibiotic resistance ~~the vector having at least one of the following characteristics:~~

(i) ~~when it is present in *Lactococcus lactis* strain FA4-1-1 (DSM 12086) having an amber mutation in the *pyrF* gene that is suppressible by the suppressor, it permits said strain to grow at 30°C at a doubling time of at the most 100 minutes in a minimal medium not containing pyrimidine sources;~~

(ii) ~~when it is present in a strain of *Lactococcus lactis* FH CY 1 that has an amber mutation in the *pyrF* gene (strain CHCC41-46, DSM 12109), the amber mutation being suppressible by the suppressor, it permits the strain to acidify milk under identical conditions at essentially the same rate of that of the parent strain (FH CY 1, DSM 12087);~~

(iii) ~~it permits the *Lactococcus lactis* FA4-1-1 strain to grow at 30°C in a minimal medium not containing pyrimidine sources at a doubling time which is less than that for the *Lactococcus lactis* strain DN209 transformed with the vector pFG1-1 (DSM 12088), the pFG1-1 vector comprising a gene coding for a suppressor that is capable of suppressing the amber mutation in the DN209 strain, the transformed DN209 strain growing under conditions identical to those for the FA4-1-1 strain.~~

2. (Currently amended) A vector according to claim 41 which has at least two of the characteristics (i) to (iii).

3. (Currently amended) A vector according to claim 41 which has the characteristics (i) to (iii).

4. (Currently Amended) A vector according to claim 1 wherein the gene coding for the suppressor is ~~derived~~ obtained from the chromosome of a lactic acid bacterium.

5. (Original) A vector according to claim 4 wherein the gene coding for a suppressor is under the control of a regulatable promoter.
6. (Currently Amended) A vector according to claim 5 wherein the regulatable promoter is a heterologous promoter ~~not naturally related to the gene~~.
7. (Original) A vector according to claim 1 wherein the amber suppressor results from at least one change of nucleotide in an anticodon.
8. (Original) A vector according to claim 7 wherein the suppressor has two or three changes of nucleotide.
9. (Original) A vector according to claim 1 wherein the suppressor is a suppressor selected from the group consisting of a *supD*, *supE*, *supF*, *supP*, *supU* and a *supZ* suppressor.
10. (Currently amended) A vector according to claim 1 wherein the replicon is ~~derived~~ obtained from a *Lactococcus lactis* plasmid.
11. (Original) A vector according to claim 1 that comprises at least one unique restriction site.
12. (Original) A vector according to claim 1 that comprises a multiple cloning site.
13. (Original) A vector according to claim 1 which is a theta-replicating plasmid.
14. (Original) A vector according to claim 1 which is stably maintained for at least 35 generations in a lactic acid bacterium cultivated in a medium not containing pyrimidine sources.
15. (Currently Amended) A vector according to claim 1 which is selected from the group consisting of pFG 100 deposited under the accession No. DSM 12091, ~~a mutant, variant or derivative of pFG 100~~, pFG200 deposited under the accession No. DSM 12108 ~~and a mutant, variant or derivative of pFG200~~, ~~said mutants, variants or derivatives essentially having the characteristics of the respective vector from which they are derived~~.
16. (Currently Amended) A vector according to claim 1 which further comprises a gene coding for a desired gene product.

17. (Original) A vector according to claim 16 wherein the gene product is a peptidase selected from the group consisting of lysine-aminopeptidase, glutamyl-aminopeptidase, cysteine-aminopeptidase, iminopeptidase, X-prolyl-dipeptidyl aminopeptidase, endopeptidase, dipeptidase and tripeptidase.

18. (Original) A vector according to claim 16 wherein the gene product confers bacteriophage resistance to a lactic acid bacterial host cell.

19. (Original) A vector according to claim 16 wherein the gene product is a bacteriophage lysin.

20. (Currently Amended) A vector according to claim 19 wherein the gene coding for the bacteriophage lysin is ~~derived~~ obtained from the bacteriophage ϕ vML3 as contained in DN209/pFG7 deposited under the accession No. DSM 12089.

21. (Currently Amended) A vector according to claim 16 wherein the gene product is ~~involved in a part of the~~ nisin synthesis pathway or nisin resistance.

22. (Previously Presented) A lactic acid bacterium comprising a vector according to claim 1.

23. (Original) A lactic acid bacterium according to claim 22 that comprises an amber mutation being suppressible by the nonsense amber suppressor.

24. (Original) A lactic acid bacterium according to claim 23 wherein the amber mutation is located on a replicon different from the one containing the gene coding for the nonsense suppressor.

25. (Original) A lactic acid bacterium according to claim 22 wherein the suppressor is one suppressing a nonsense mutation which in the absence of a nonsense suppressor capable of suppressing the mutation, confers auxotrophy.

26. (Original) A lactic acid bacterium according to claim 25 wherein the nonsense mutation is in a gene involved in the synthesis of pyrimidine nucleotides.

27. (Original) A lactic acid bacterium according to claim 26 wherein the nonsense mutation is in a *pyr* gene.
28. (Original) A lactic acid bacterium according to claim 22 which is selected from the group consisting of a *Lactococcus* sp., *Streptococcus* sp., *Lactobacil/us* sp., *Leuconostoc* sp., *Pediococcus* sp. and *Bifidobacterium* sp.
29. (Original) A lactic acid bacterium according to claim 28 which is *Lactococcus lactis*.
30. (Original) A lactic acid bacterium according to claim 29 which is *Lactococcus lactis* subsp. *lactis* strain FA4-1-1 containing pFG100, deposited under the accession No. DSM 12091 or *Lactococcus lactis* subsp. *lactis* strain CHCC4146 containing pFG200, deposited under the accession No. DSM 12108.
31. (Original) A lactic acid bacterium according to claim 22 wherein the vector is stably maintained for at least 35 generations when it is cultivated in a medium not containing pyrimidine sources.
32. (Original) An isolated pure culture of a lactic acid bacterium according to claim 22.
33. (Original) A composition comprising an isolated pure culture of a lactic acid bacterium as defined in claim 32, and a carrier.
34. (Currently amended) A composition according to claim 33 containing at least 10^5 colony forming units of the lactic acid bacterium per g-gram composition.
35. (Currently Amended) ~~A method of using~~ Use of a composition as defined in claim 33 as a starter culture in the preparation of a product selected from the group consisting of a dairy flavour, a product for cheese flavouring, a food product and a feed product.
36. (Original) A method of stably maintaining a vector according to claim 1 in lactic acid bacterial host cells growing in a particular environment, comprising providing said host cells as nonsense mutant cells having lost the capability of growing in said environment, and transformed with the vector according to claim 1 containing a nonsense suppressor gene encoding a gene product restoring the capability of the nonsense mutant cells to grow in said environment whereby, if the vector is lost from the lactic acid bacterial cells, the cells will not grow.

37. (Original) A method according to claim 36 wherein the nonsense mutant cells having lost the capability to grow are auxotrophic cells.

38. (Original) A method according to claim 37 wherein the nonsense mutant cells have a mutation in a gene involved in the synthesis of nucleotides.

39. (Original) A method according to claim 38 wherein the lactic acid bacterial host cells are *pyr* mutants.

40. (Original) A method according to claim 36 wherein the environment is a material selected from the group consisting of milk, a vegetable material, a meat product, a must, a fruit juice, a wine, a dough and a batter.

41. (New) The recombinant vector of claim 1, wherein the vector comprises at least one of the following characteristics:

(i) when it is present in *Lactococcus lactis* strain FA4-1-1 (DSM 12086) having an amber mutation in the *pyrF* gene that is suppressible by the suppressor, it permits said strain to grow at 30°C at a doubling time of at the most 100 minutes in a minimal medium not containing pyrimidine sources;

(ii) when it is present in a strain of *Lactococcus lactis* CHCC41 46 (DSM 12109) having an amber mutation in the *pyrF* gene, the amber mutation being suppressible by the suppressor, it permits the strain to have an acidification rate that, in a comparative experiment with the parent strain *Lactococcus lactis* FH CY-1 (DSM 12087), results in a Δ pH of at the most 1.0 after about 3 hours of cultivation;

(iii) it permits the *Lactococcus lactis* FA4-1-1 strain to grow at 30°C in a minimal medium not containing pyrimidine sources at a doubling time which is less than that for the *Lactococcus lactis* strain DN209 transformed with the vector pFG1 .1 (DSM 12088), the pFG 1.1 vector comprising a gene coding for a suppressor that is capable of suppressing the amber mutation in the DN209 strain, the transformed DN209 strain growing under conditions identical to those for the FA4-1-1 strain.

42. (New) The recombinant vector of claim 1, wherein component (a) consists essentially of lactic acid bacterial DNA.